The IL-6-174C/G polymorphism analysis in Ukrainian residents as prospects for biomedical and pharmacogenetic uses

It is reliably determined that the presence of the G allele in the 174C/G polymorphic region of the IL-6 gene promoter and a higher level of IL-6 are more commonly observed among patients suffering from various metabolic disorders and obesity, malignant tumors, type 2 diabetes, periodontitis, oxidative stress. Over time, muscle damage and inflammation processes develop.

Aim. To study the frequency of the IL-6-174C/G single nucleotide polymorphism in the Ukrainian population.

Materials and methods. Buccal swab samples for the DNA analysis were collected from 102 healthy volunteers (48 males, 54 females, Ukrainian residents, predominantly ethnic Ukrainians). Genotyping to determine the IL-6-174C/G polymorphism was performed on DNA samples from the buccal epithelium using the polymerase chain reaction followed by RFLP. Control of the genotype distribution for compliance with the Hardy–Weinberg equilibrium was performed using the $\chi^2$ criterion.

Results and discussion. The distribution of genotypes of the IL-6-174C/G polymorphism in the Ukrainian population samples was as follows: CC – 46 %, CG – 49 %, and GG – 5 % of residents. The frequency of the IL-6-174C/G polymorphism allele in the population was $p_G$ – 0.71 and $q_G$ – 0.29. The population structure did not deviate from the Hardy–Weinberg equilibrium since there was no difference between the theoretically expected and actual frequencies of three genotypes.

Conclusion. The data obtained demonstrates the presence of the IL-6-174C/G polymorphism in the Ukrainian population.

Key words: IL-6 gene; IL-6-174C/G; polymorphism; Ukraine; allele frequency; Hardy–Weinberg equilibrium

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Introduction. The IL-6 gene is presented in 7p21 chromosome and consists of four introns and five exons [1]. As the single nucleotide polymorphism can affect both intron and exon areas of a gene, the structural changes can lead both to a change of gene expression and a change in the IL-6 activity. Replacement of cytosine (C) with guanine (G) in the promoter gene region, namely in the 5′ flanking region in position 174 is the well-studied gene polymorphism. In this case, the change of the IL-6 gene transcriptional activity in response to some regulatory factors was described. It is known that people with the G allele have higher IL-6 concentrations in the blood compared to people with the C allele, which is an alternative for this position [2]. The relationship between the IL-6 gene polymorphism, the level of the corresponding pro-inflammatory cytokine and clinically significant human traits is still not clear. This may be connected with the fact that IL-6 has both pro-inflammatory and anti-inflammatory features.

It is shown that polymorphism in the IL-6-174C/G promoter is associated with metabolic disorders and obesity [3], malignant tumors [4–6], type 2 diabetes [7], hearing loss [8], oxidative stress with subsequent muscle injuries and inflammation processes [9], etc. In particular, some authors discovered relations between C allele findings and certain diseases: an active form of toxoplasmosis.
with progressive toxoplasmic retinochoroiditis [10], type 2 diabetes [11], sepsis [12]; diseases having a low survival rate: neuroblastoma [6], endometrium adenocarcinoma [13], squamous cell carcinoma of the oral cavity [14], complicated breast cancer [5]. An increased frequency of paranoic schizophrenia, in which a failure of the immune system is observed [15]. On the other hand, the G allele is associated with coronary artery disease and hypertensive heart disease [16].

At the same time, there was either no information about associations of the IL-6-174C/G polymorphism or that information was contradictory. In particular, during experimentally induced human endotoxemia caused by intravenous administration of lipopolysaccharide, the genotype of the participants did not depend on changes in the level of IL-6 in plasma [17]. There was also no dependence between the IL-6 gene polymorphism and the risk of multiple myeloma [18], late Alzheimer’s disease [19], type 2 diabetes [20], head and neck cancer [4]. Some contradictory information concerning the protective CC genotype is present: among Greeks suffering from type 2 diabetes [21].

Materials and methods. In order to study the population distribution of the IL-6-174C/G polymorphism, the sample consisted of Ukrainian residents, predominantly ethnic Ukrainians, was selected. Buccal swab samples for the DNA analysis were collected from 102 healthy volunteers (48 males, 54 females) who were not relatives. The material was collected in accordance with ethical standards of work under the Helsinki Declaration (World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects). Genotyping to determine the IL-6-174C/G polymorphism was performed on DNA samples from the buccal epithelium using the polymerase chain reaction followed by RFLP [22]. DNA was isolated from buccal epithelium samples of the subjects using a Chelex-100 ion-exchange resin [23]. The allelic state of the IL-6 gene was determined by a 174C/G single nucleotide replacement (rs2069840) according to the method [24]. Amplification was performed using a “Tercyc” DNA amplifier (DNA-Technology). To amplify a fragment of the IL-6 gene containing a polymorphic site (174C/G), the forward primer TGAACCTCAAGCTTACTTCTTTGT and reverse primer AAATAGGTITTTGAGGGCCATG were used [25]. Restriction of amplification products was performed using Hin1II endonuclease (NlaIII) (MBI Fermentas, Lithuania). The restriction products were analyzed using electrophoresis in 2 % agarose gel. 1×TBE was used as a electrophoretic buffer. DNA pUC19 hydrolyzed by MspI endonuclease (MBI Fermentas, Lithuania) was used as a molecular weight marker. The visualization of amplification and restriction products was performed by staining a gel with ethidium bromide and photographing on a transilluminator in ultraviolet light. The restriction fragment sizing 164 bps corresponded to the C allele of the 174C/G variant of the IL-6 gene, while two restriction fragments sizing 112 and 52 bps, respectively, corresponded to the G allele.

The frequencies of alleles (p and q) were calculated according to the results of genotyping:

\[ p_c = \frac{2CC + CG}{2N} \]

\[ q_c = \frac{2GG + CG}{2N} \]

where \( N \) is the number of the study participants.

The frequencies of alleles (C and G) were calculated separately for males and females. The average weighted frequencies for the corresponding alleles were also calculated; they were \( p_c = 0.71 \) and \( q_c = 0.29 \), respectively (Table 2).

According to the frequencies of actual alleles, the frequencies of the corresponding genotypes were calculated based on the Hardy–Weinberg proportion (Table 3). The theoretically expected frequencies of genotypes calculated using Hardy–Weinberg equations were not statistically significantly different from the observed ones (Table 4). That allows us to conclude that there is an equilibrium in the IL-6-174C/G polymorphism in the sample of the Ukrainian population.

The frequencies of indicated alleles were studied in a number of populations. Studies have shown that world population differs by frequency of these alleles.

### Table 1

**Distribution of the IL-6-174C/G polymorphism genotypes**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Males, N</th>
<th>Females, N</th>
<th>Total, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>19</td>
<td>28</td>
<td>47 (46)</td>
</tr>
<tr>
<td>CG</td>
<td>26</td>
<td>24</td>
<td>50 (49)</td>
</tr>
<tr>
<td>GG</td>
<td>3</td>
<td>2</td>
<td>5 (5)</td>
</tr>
</tbody>
</table>

*Statistics: \( \chi^2 = 1.656, df = 2, p > 0.05. \)*

*Note. \( \chi^2 \) – Pearson criterion, df – degree of freedom, \( p \) – level of significance.*

### Table 2

**The frequencies of C and G alleles of the IL-6-174C/G polymorphism**

<table>
<thead>
<tr>
<th>Alleles</th>
<th>C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>Females</td>
<td>0.74</td>
<td>0.26</td>
</tr>
<tr>
<td>Total</td>
<td>0.71</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Table 3
Genotypes frequencies in the IL-6-174C/G polymorphism

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>0.45</td>
<td>0.55</td>
<td>0.50</td>
</tr>
<tr>
<td>CG</td>
<td>0.44</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>GG</td>
<td>0.11</td>
<td>0.07</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 4
The theoretically expected frequencies of the IL-6-174C/G polymorphism genotypes in the population

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>The theoretically expected frequencies of genotypes</th>
<th>The actual frequencies of genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>CG</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>GG</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

Statistics: $\chi^2 = 1.382$, df = 2, $p > 0.05$.

In particular, in Brazil one of the studies showed that the G allele was major, meanwhile the C allele was minor. As a result, in healthy people $p_C = 0.92$ and $p_G = 0.08$; people with the CC genotype – 6 %, GG – 89.2 % and CG – 4.8 %. In the group of patients with toxoplasmatic retinochoroiditis the corresponding frequencies were different: $p_C = 0.83$ and $p_G = 0.17$, CC homozygotes – 6.2 %, GG homozygotes – 71.1 % and CG heterozygotes – 22.7 % [11]. There are data obtained from the study of Greece residents where a sample of 393 patients with type 2 diabetes was studied: the distribution of genotypes was as follows: GG – 49.1 %, GC – 26.8 % and CC – 24.1 % of people; genotype frequencies had no gender differences [21]. In India, the frequencies of CC homozygotes, GG homozygotes and CG heterozygotes among the healthy population were 4.9 %, 69.7 %, and 25.4 %; the frequencies of C and G alleles – 0.18 and 0.82, respectively. Similar indicators in patients with squamous cell carcinoma of the oral cavity were 7.7 %, 55.2 % and 37.1 %; the frequencies of C and G alleles – 0.26 and 0.74, respectively [14].

It is obvious that C and G alleles can significantly vary in different groups of polyethnic populations. For instance, in Malaysia, where three main ethnic groups are Malayan, Chinese and Hindu, the frequency of G allele in the general population is 0.91, C allele – 0.09. At the same time, in the Malayan group the frequency of G allele is 0.04, among Hindu – 0.19. However, this allele was not detected in the Chinese population [25]. Previously, the study of the IL-6-174C/G polymorphism, was conducted in Ukraine and included cancer patients and healthy individuals. In that work, in contrast to our current study, the G allele was predominant in the control group of healthy individuals [26]. However, this fact may be explained by polyethnicity in Ukraine. Due to this, we can observe the vast variation in the ethnic composition in different parts of country, and therefore, we can suggest the genetic polymorphism effect in different genes, theoretically not excluding the IL-6 gene. The above-mentioned study, which involved cancer patients and healthy individuals, was conducted in the southern part of Ukraine, while the volunteers of our current study were mainly residents of the eastern part of Ukraine. Moreover, the Slavic population from different, but neighboring countries may be very close in ethnic composition, unlike those located in the same country, but geographically distant from each other. Thus, the general conclusion is that the gene frequencies studied and different frequencies of genotypes indicate the population distinctions in the IL-6-174C/G polymorphism.

Conclusions and prospects for further research. Our research has demonstrated that the distribution of genotypes of the IL-6-174C/G polymorphism in the Ukrainian population sample is as follows: CC – 46 %, CG – 49 % and GG – 5 % of residents. The frequency of the IL-6-174C/G polymorphism alleles in the population was $p_C = 0.71$ and $q_G = 0.29$. The population structure does not deviate from the Hardy–Weinberg equilibrium since there is no difference between the theoretically expected and actual frequencies of three genotypes.

Conflict of interests: the authors declare that they have no conflicts of interest.

REFERENCES


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